POTENCY OF BELIMBING WULUH (AVERRHOA BILIMBI) AS ANTIOXIDANT AND TYROSINASE INHIBITOR FOR SKIN WHITENING PRODUCTS

By NINING SUGIHARTINI

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Research Article

POTENCY OF BELIMBING WULUH (*AVERRHOA BILIMBI*) AS ANTIOXIDANT AND TYROSINASE INHIBITOR FOR SKIN WHITENING PRODUCTS

Ririn Suharsanti [1]*, Nining Sugihartini [2], Endang Lukitaningsih [3], Muhammad Ryan Radix Rahardhian [1]

[1] *Sekolah Tinggi Ilmu Farmasi"Yayasan Pharmasi Semarang", Jl. Letjend sarwo edie wibowo Km 1 Plamongansari Semarang, INDONESIA.*

[2] *Ahmad Dahlan University, Jalan Prof. Dr. Soepomo, S.H. Janturan Yogyakarta, INDONESIA.*

[3] *Gadjah Mada University, Jl. Sekip Utara* Yogyakarta, INDONESIA.

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ABSTRACT

Hyperpigmentation is an event that occurs due to excessive production of skin pigments, where the presence of melanin. Belimbing wuluh as Indonesian plant is rich compounds that function as antioxidants and tyrosinase inhibitor. The aim this research to test the potency of belimbing wuluh extracts as antioxidant and tyrosinase inhibitors for active ingredients whitening for skin. The method used in the study is experimental laboratory with some testing among others test for antioxidant activity with DPPH. Rutin as a standard for antioxidant activity. Tyrosinase inhibitor activity using tyrosinase from mushroom as enzym and L-tyrosine as a substrate with kojic acid as a standard. The IC_{50} value of antioxidant activity of belimbing wuluh fruits extract is 2331.69±339.09 µg/ml and 320.76±79.54 µg/ml from leaves extract. The IC_{50} value of antioxidant activity greater than rutin 5.92 µg/ml. Tyrosinase inhibitor activity can be seen from IC_{50} values is 186.85±9.37 µg/ml from fruits extract and 150.57±6.25 µg/ml from leaves extract. The IC_{50} value of tyrosinase inhibitor greater than kojic acid 16.68 µg/ml. Our findings showed that *belimbing wuluh leaves extract better than fruits extract. These results suggest that belimbing wuluh is a natural ingredient that has potential to be used in halal skin whitening product.*

KEYWORDS: *Antioxidant, Belimbing Wuluh, Tyrosinase Inhibitor.*

INTRODUCTION

Hyperpigmentation is one of the skin damage characterized by the appearance of dark spots on the skin. The appearance of dark spots on the skin is caused by the increase of melanin substances. Melanin substances do have a role in the color of a person's skin pigments as excessive production of melanin will cause dark spots on the skin. Various causes are considered to be the trigger for the appearance of spots on the skin. One of them, hyperpigmentation occurs because of exposure to UV rays from the sun [1]. Melanin is formed through a pathway called melanogenesis with the help of the tyrosinase enzyme. This formation is affected by exposure to UV light with the mechanism of melanocyte proliferation. Hyperpoliferation of melanocytes results in over production of melanin which can cause hyperpigmentation of the skin which causes black spots and potentially melanoma [2]. This makes the prevention important to do one of them by controlling the enzyme tyrosinase. One of Indonesia's tropical country plants is belimbing wuluh. Belimbing wuluh (*Averrhoa bilimbi*) is known to contain efficacious compounds such as phenolics such as flavonoids [3]. Belimbing wuluh fruits contains total phenol compounds of 65 mg GAE/g extract. Phenolic compounds and flavonoids have activities as antioxidants [4, 5]. The bond of flavonoids with copper and their antioxidant effects have been reported to play a role in inhibiting the action of the enzyme tyrosinase [6]. Never reported about antioxidant activity and inhibitory activity of tyrosinase belimbing wuluh leaves and fruits. The purpose of this study was to know the potential of belimbing wuluh as an antioxidant and tyrosinase inhibitor in different parts.

***Corresponding author:**
Ririn Suharsanti
Sekolah Tinggi Ilmu Farmasi"Yayasan Pharmasi Semarang", Jl. Letjend sarwo edie wibowo Km 1 Plamongansari Semarang, INDONESIA.
** E-Mail: ririnsuharsanti@stifar.ac.id*

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MATERIALS AND METHODS

Materials:

Fresh of *Belimbing Wuluh* leaves and fruits were determination by a botanist at laboratory of pharmaceutical biology Sekolah Tinggi Ilmu Farmasi "Yayasan Pharmasi Semarang" (Stifar). Chemical reagents: ethanol, rutin, DPPH, kojic acid, tyrosinase from mushroom as enzym and L-tyrosine as a substrate were supplied by Sigma–Aldrich (St. Louis, MO,

USA). All chemicals and reagents used in the study were of analytical grade.

Methods:

1. Extraction of belimbing wuluh:

The 200 g dried [7] and powdered belimbing wuluh leaves and fruits were remaserated with 96% ethanol for 3 days at room temperature. Liquid extract was evaporated at 60°C, 100 RPM, to viscous extract.

2. Determination of antioxidant activity:

The antioxidant activity of belimbing wuluh's leaves and fruits extract were evaluated using 2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. Ten mg of belimbing wuluh leaves and fruits extract diluted with methanol in 10 mL flask. From each extract solution, a series of concentrations was made from 100 µg/mL to 5 µg/ mL. One mL of this solution added with 1 mL DPPH 0,4 mM and methanol until 5 mL in volumetric flask. The contents were mixed and incubated for 30 minute as operating time [8]. Each series solution of extract measured at a maximum wavelength of 517 nm as λmax with Vis spectrophotometer [8]. Rutin standard antioxidant is used as a comparison made with concentration 2 µg/mL to 10 µg/ mL. IC_{50} values denote the concentration of sample which is required to scavenge 50% of DPPH free radicals [9, 10].

3. Determination of of enzyme inhibition activity:

Each extract test for inhibition of tyrosinase activity used Lai method [11] with modification, 500 µL of Ea extract with various concentrations mixed in 1000 µL of phosphate buffer sol pH 6.8 was added with 500 µL L-tyrosine (2.5 µM) in a phosphate buffer pH 6.8. The solution mixture was incubated in a dark place for 10 minutes. Then, 500 µL of mushroom tyrosinase solution (25 KU) was added to the mixture, which was then incubated for 30 minutes at room temperature. Absorbance was measured with spectrophotometer at 480 nm. The concentration of kojic acid was made at a concentration of 10-20 µg/mL and treated with the same sample. For blanks, complete analytical procedures are followed, including all chemica and solvents, but no samples are added inhibitory effects of the extracts were expressed as the inhibitor concentration causing a 50 % loss of enzyme activity (IC_{50}) [12, 13].

RESULTS AND DISCUSSION

Determination of antioxidant activity:

The method used in testing antioxidant activity is the method of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical uptake because it is a simple, fast, easy method, and uses samples in small amounts with a short time [2]. The measurement of antioxidant activity was carried out using UV-Vis spectrophotometry at a wavelength of 517 nm which is the maximum wavelength for DPPH. DPPH compound is a molecule that contains an unstable nitrogen free radical compound which can bind hydrogen ions so that it is used to test antioxidant activity. The presence of antioxidant compounds from the sample resulted in discoloration in the DPPH solution in methanol which was originally concentrated violet to pale yellow [14]. This color change occurs because DPPH is reduced so that electrons become paired. In this study routine is used as a comparison. The activity as an antioxidant possessed by most flavonoids is caused by the presence phenolic hydroxy group in its molecular structure. When these compounds react with free radicals, they form new radicals stabilized by the resonant effect of the aromatic nucleus [15]. Percent reduction in free radicals from leaves and fruits extracts is shown in Figure 1.

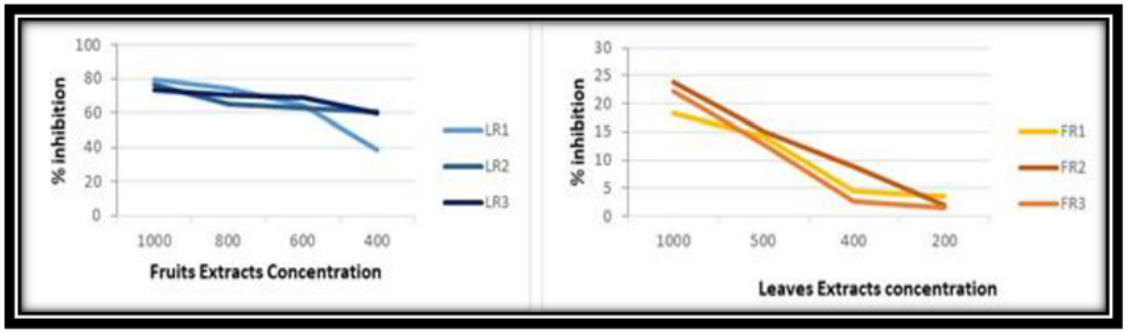

Fig. 1: The % inhibition antioxidant value of belimbing wuluh

Leaves are more able to inhibit free radicals compared to fruits. The IC_{50} (Inhibitor concentration) by the 50% centration test which can reduce free radicals. The smaller the IC_{50} value, the higher the activity of free radicals. The working principle of free radicals can be muted [12, 14]. The IC_{50} value of antioxidant activity of fruits and leaves extracts greater than rutin as standard.

According to the division of antioxidant categories Blois [16], a natural material (raw material) that has IC_{50} less than 50 µg / mL can be categorized as a very strong antioxidant, 50-100 µg / mL as a powerful antioxidant, 101-150 µg / mL as a moderate antioxidant and if more than 159 µg / mL are categorized as a weak antioxidant. IC_{50} The test results showed that the leaves and fruits extracts included in the category of weak antioxidants. When compared with the rutine, the IC_{50} value of extracts is much greater than the rutin (30.18 µg / mL). This can happen because the extract is not a pure compound like rutin.

Determination of tyrosinase inhibitor:

Tyrosinase inhibition activity test was carried out by the lai method [11] at optimum testing conditions, namely maximum wavelength of 480 nm, incubation time of 30 minutes, L-tyrosine substrate concentration of 2.5 mM, enzyme concentration of 25 KU, and temperature 25-30 ° C. Sample control solutions were made for each test as a comparison of sample absorption data with and without enzymes, while blank

solutions were made as a correction factor and using kojic acid as a positive control. The result of tyrosinase inhibition by leaves and fruits extracts can be seen in Figure 2.

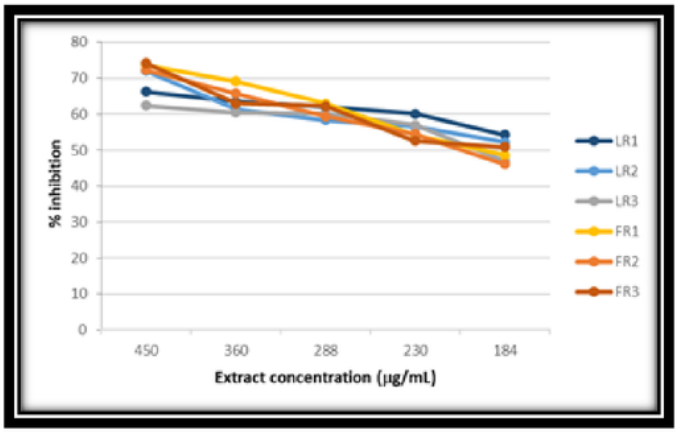

Fig. 2: The % inhibition tyrosinase value of belimbing wuluh

Belimbing wuluh's leaves have better ability than fruits. These results are in line with the results of testing the antioxidant content that has been done before and can be seen in Figure 3. Antioxidants as free radical catchers can inhibit tyrosinase enzyme activity and inhibit the transcription of tyrosinase genes [17]. Can Tyrosinase inhibitor activity of sample be seen from IC_{50} values greater than kojic acid (16.68 µg / mL) as standard. When referring to the classification of activities according to [18, 19] leaves and fruits belimbing wuluh extracts were classified as inactive because the IC_{50} value was <1000 ppm.

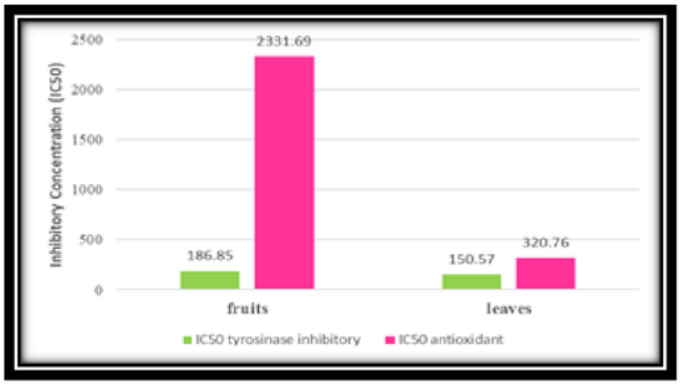

Fig. 3: The IC_{50} antioxidant and inhibitor tyrosinase values of belimbing wuluh

CONCLUSIONS

Our findings showed that belimbing wuluh leaves extract better than fruits extract. These results suggest that belimbing wuluh is a natural ingredient that has potential to be used in halal skin whitening product.

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